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Characterization of the 9-Cis-Epoxycarotenoid Dioxygenase Gene Family and the Regulation of Abscisic Acid Biosynthesis in Avocado[1]

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Avocado (*Persea americana* Mill. cv Lula) is a climacteric fruit that exhibits a rise in ethylene as the fruit ripens. This rise in ethylene is followed by an increase in abscisic acid (ABA), with the highest level occurring just after the peak in ethylene production. ABA is synthesized from the cleavage of carotenoid precursors. The cleavage of carotenoid precursors produces xanthoxin, which can subsequently be converted into ABA via ABA-aldehyde. Indirect evidence indicates that the cleavage reaction, catalyzed by 9-cis-epoxycarotenoid dioxygenase (NCED), is the regulatory step in ABA synthesis. Three genes encoding NCED cleavage-like enzymes were cloned from avocado fruit. Two genes, *PaNCED1* and *PaNCED3*, were strongly induced as the fruit ripened. The other gene, *PaNCED2*, was constitutively expressed during fruit ripening, as well as in leaves. This gene lacks a predicted chloroplast transit peptide. It is therefore unlikely to be involved in ABA biosynthesis. *PaNCED1* was induced by water stress, but expression of *PaNCED3* was not detectable in dehydrated leaves. Recombinant PaNCED1 and PaNCED3 were capable of in vitro cleavage of 9-cis-xanthophylls into xanthoxin and C_{25}-apocarotenoids, but PaNCED2 was not. Taken together, the results indicate that ABA biosynthesis in avocado is regulated at the level of carotenoid cleavage.